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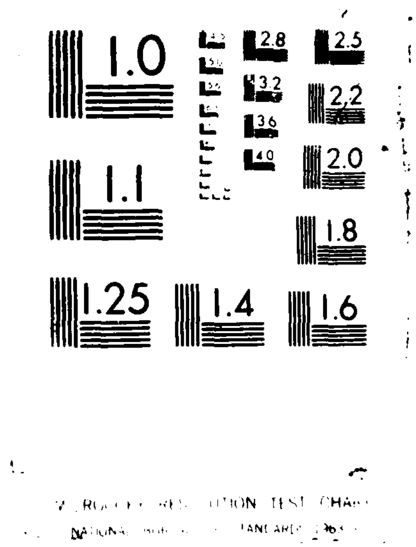
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Review of Cytoskeleton Research in Cell Differentiation and Development

Claire E. Zomzely-Neurath

10 September 1987

U.S. Office of Naval Research, London

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Papers given at this conference, held in April 1987 in Granada, Spain, are reviewed. The papers focused on the analysis of the assembly dynamics of microtubules, intermediate filaments, and actin filaments to provide the structural basis of the role played by the cytoskeleton in differentiating a variety of cell systems, early embryogenesis, and to the biological and genetic aspects of cytoplasmic organization.

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CONTENTS

REVIEW OF THE CYTOSKELETON RESEARCH IN CELL DIFFERENTIATION AND DEVELOPMENT

1 INTRODUCTION

The first international symposium on the cytoskelton in cell differentiation and development was held in Granada, Spain, from 21 through 25 April 1987 at the Faculty of Medicine, University of Granada. This specialized conference was organized jointly by J. Arechaga (Department of Cellular Biology, University of Granada) and R.B. Maccioni (University of Colorado Health Sciences Center, Denver). This joint venture was reflected in the fact that of the total of 95 participants, 38 percent were from Spain and 26 percent from the US. The balance of attendees represented eight European countries as well as Chile, Israel, and South Africa.

The elucidation of the structural-functional aspects of early development and cellular differentiation are among the most challenging problems in modern biology. The developmental aspect of the cytoskeleton is clearly one of the relevant aspects of this rapidly growing research field. Research on cytoskeletal structure and organization has been a rewarding area of investigation, exhibiting an explosive growth of scientific ideas and information during the past 15 years which has contributed to an understanding of the biological complexity of cytoplasmic organization and the intracellular dynamics. Especially, multidisciplinary approaches have impacted and stimulated research in many fields of biological sciences including cell biology, biochemistry, and molecular and developmental biology. Thus, within the framework of the perspective of epistemologists the organization and assembly of the cytoskeleton constitutes a major conceptual scheme of modern biology.

The detailed cellular and genetic aspects of embryogenesis were discussed in several plenary lectures while the developmental aspects of cancer research were the subject of a symposium session. The structure and regulation of microtubules and their internal organization in differentiating cells and embryos was also a major topic of the symposia. Further analysis of these regulatory aspects in the light of microtubule-associated proteins (MAP's) and the interactions of cytoskeletal components were also developed in the various sessions of the conference. Besides the control of the activity and organization of the cytoskelton by multiple protein factors and ligands, the manner in which gene expression for different cytoskeletal components is regulated is also a crucial aspect of the analysis of the cytoskeleton in embryonic development. Developmental expression of tubulin, MAP's, and intermediate filaments as well as the molecular genetics of embryogenesis were also discussed throughout the meeting. Due to the contribution of cytoskelton research to developmental neurosciences, a special session was devoted to analyzing the organization of the cytoskeleton in differentiating neurons, glia, and neuritelike processes.

Since it is not possible to present all the topics covered in this intensive and specialized conference, summaries of selected subjects will be presented in this report. The symposium proceedings, including full papers and selected short reports, will be published in about 6 months by IRL Press Limited of Oxford, UK, on behalf of The International Council of Scientific Unions (ICSU Press).

2 MICROTUBULE ASSEMBLY AND REGULATION

The biochemical aspects of the regulation of microtubule assembly were discussed by R.B. Maccioni (University of Colorado Health Sciences Center, Denver). Protein-chemical and structural studies have provided a view of tubulin as a macromolecule containing spatially discrete sequences that constitute functionally different domains involved in self-association, and interaction with MAP's and other regulatory ligands. The 4-kilodalton (kDa) carboxyl terminal moiety of tubulin subunits located in the outer surface of the microtubule (MT) has been shown to play a major role in modulating its assembly into MT and the tubulin interaction with colchicine. Maccioni and his group, in collaboration with J. Arechaga (Department of Cellular Biology, University of Granada, Spain), have investigated the regulation of tubulin assembly and the interaction with MAP's on the basis of an integrated approach using limited proteolysis, assembly analysis, and binding studies.

These investigators found that the removal of the acidic moiety around the C-terminus (6 to 8 amino acids) of alpha and beta tubulin by carboxypeptidase Y (C-tubulin) did not relieve the modulatory effect of the C-terminal domain and the usual need of MAP's for MT polymerization as does removal of the 4-kDa segment. Treatment with carboxypeptidase produced a decay of tubulin assembly. Polymers assembled from C-tubulin and MAP's contained MAP's suggesting that the last few C-terminal residues are not directly involved in the selective interaction with MAP's. In both cases assembly decay was associated with important changes in conformation. One-site subtilisin digestion produced S-tubulin with an increased propensity to self-assemble as compared with tubulin. Cleavage with V-8 protease also resulted in removal of a 4-kDa C-terminal fragment (V-tubulin) with a concomitant stimulation of assembly. The critical concentration for assembly of V-tubulin which lacks the 4-kDa peptide in the beta subunit was threefold higher than that of S-tubulin where both subunits are cleaved, suggesting that tubulin may also contribute to the modulatory effect. No MAP's incorporation into subtilisin polymers and a partial incorporation into V-tubulin polymer was observed. The substucture of the tubulin's regulatory domain was further examined by binding of ^{3}H-acetylated peptides from the variable region of C-terminal domain alpha (430 to 441) and beta (422 to 433) to MAP's. The binding data showed a preferential interaction of beta peptide with MAP-2 and Tau as analyzed by Airfuge ultracentrifugation and Sepharose chromatography. The alpha (430-441) peptide interacted with Tau with a higher affinity than MAP-2. These studies support Maccioni's hypothesis that the C-terminal domain hinders the interactions responsible for tubulin assembly and that cleavage at or near the 4-kDa end is critical to relieve this hindering effect.

A report on phase dynamics at microtubule ends was presented by L. Wilson (Department of Biological Sciences, University of California, Santa Barbara). Wilson and his group examined the length dynamics of MAP-rich and MAP-depleted bovine brain microtubules at polymer mass steady state. In both preparations, the microtubules exhibited length redistributions shortly after attaining polymer mass steady state. With time, however, both populations relaxed to a state in which no further changes in length distributions could be detected. Shearing the microtubules or diluting the microtubule suspensions temporarily increased the extent to which microtubule length redistributions occurred, but again the microtubules relaxed to a state in which changes in the polymer length distributions could not be detected. Under steady-state conditions of constant polymer mass and stable microtubule length distribution, both MAP-rich and MAP-depleted microtubules exhibited behavior consistent with treadmilling. MAP's strongly suppressed the magnitude of the length redistributions and also the treadmilling rates. These data suggest, according to Wilson, that the inherent tendency of microtubules *in vitro* is to relax to a steady state in which net changes in the microtubule length distributions do not occur. If the basis of the observed length redistributions is the spontaneous loss and regain of GTP-tubulin ("GTP caps") at microtubule ends, then in order to account for stable length distributions the microtubules must reside in the capped state far longer than in the uncapped state, and uncapped microtubule ends must be recapped rapidly. If this is also true for microtubules in cells, then there must be a mechanism in cells for depolymerizing the microtubules actively, perhaps by removing or suppressing reformation of GTP caps.

The role of posttranslational tyrosination in cytoskeletal differentiation was discussed by G.G. Gundersen (Department of Biology, University of California, Los Angeles). In most proliferating cells in culture, a small proportion of the microtubules (MT) are enriched in detyrosinated (Glu) tubulin, while the majority contain predominantly tyrosinated (Tyr) tubulin. These populations are created by a cycle of detyrosination of MT's and retyrosination of monomeric tubulin. Thus, newly formed MT's contain Tyr tubulin, while long-lived MT's contain increasing levels of Glu tubulin, as found by Gundersen and his group. Whereas earlier studies failed to detect significant differences in the behavior of Tyr and Glu MT's *in vitro* , these investigators have now found that Tyr and Glu MT's do behave differently *in vivo*. In TC-7 epithelial cells, Tyr and GLu MT's exhibited nearly identical sensitivity to cold treatment; however, Glu MT's were about 30 percent more stable to nocodazole depolymerization. Similarly, if TC-7 cells were extracted under conditions that allowed depolymerization by dilution of the monomer pool, Glu MT's were about 20 percent more stable than Tyr MT's. If the Tyr MT's in the extracted cells were converted to Glu MT's by carboxypeptidase A treatment, they did not exhibit increased stability, suggesting that the stability of Glu MT's *in vivo* was not solely due to the detyrosination of MT subunits.

In a second type of experiment, Gunderson and coworkers determined whether both types of MT's were actively growing *in vivo* by examining the immunofluorescence (IF) staining of MT ends distal to the centrosome. Since the monomer pool in TC-7 cells is greater than 98 percent Tyr tubulin, growing MT's should contain only Tyr IF at their distal ends. They found that while almost all Tyr MT's were growing, all those containing elevated levels of Glu tubulin were not. These data show that Glu and Tyr MT's do exhibit distinct functional properties and suggest that the ends of Glu MT's are structurally altered to restrict subunit addition and loss. According to Gundersen, a similar modification of MT ends may be important in the cytoskeletal reorganizations that occur during differentiation. Thus, dramatic increases have been observed in the level of Glu MT's during neurite outgrowth in PC-12 cells and during fusion of L6 rat myoblasts to form myotubes.

3 DIFFERENTIATION IN MICROTUBULE-ASSOCIATED PROTEINS (MAP'S)

The topic of common and unique tubulin binding sites for MAP 2 and Tau as a mechanism for microtubule divergence was discussed by U. Littauer (Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel). Littauer reported that analysis of the derived amino acid sequence of complementary DNA (cDNA) clones shows a stringent conservation of the carboxy-terminal region of alpha-tubulin isotypes from various sources. On the other hand, beta-tubulin isotypes show a variable domain within the 15 carboxy-terminal amino acids. Littauer thinks that these differences between the various beta-tubulin isotypes may contribute to changes in tubulin binding sites towards various ligands, thus generating microtubules with different functions. This hypothesis is supported by recent experiments by Littauer and coworkers in which they have developed a specific binding assay that monitors the interaction of (^{125}I) MAP's with tubulin or its cleavage peptides. To identify the tubulin-binding domains for MAP's they have examined the binding of rat brain (^{125}I) MAP 2 or (^{125}I) Tau factors to 60 cleavage peptides derived from pig alpha- and beta-tubulin. Their results showed that MAP 2 will specifically interact with only two peptides located at the carboxy-terminus of beta-tubulin, between positions 392-445 and 416-445. Strong binding sites for Tau factors were also located at the carboxy-terminus of beta-tubulin, between positions 392-445 and 416-445. In addition, Tau factors, but not MAP 2, interacted with a peptide located near the N-terminus of alpha-tubulin between positions 1-75.

To narrow down the location of the beta-tubulin binding site that is common to MAP 2 and Tau factors, these investigators have synthesized five peptides which are homologous to the corresponding sequence from porcine or rat C-terminal region. (The peptide synthesis was carried out by H. Ponsting of the Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, West Germany.) Binding studies with the synthetic peptides suggest that amino acid residues 434-440 of beta-tubulin are crucial for the interaction of MAP 2 and Tau factors. Thus, both MAP 2 and Tau factors share one common binding site near the carboxyl terminus of beta-tubulin. According to Littauer, it also appears that this region has diverged and evolved more rapidly throughout evolution than the other constant regions of tubulin. It is possible that the binding domain in the proximity of the 3'-end of the tubulin molecule and the corresponding site on MAP's underwent molecular coevolution and thus provides a mechanism to generate new control mechanisms for microtubule assembly.

Studies on the regulation of the polymerization of microtubule proteins were reported by J. Avila (Center of Molecular Biology, Madrid, Spain). Tubulin assembly into microtubules is stimulated by different MAP's. Limited proteolysis of tubulin and MAP's to analyze the structure of these proteins may facilitate knowledge of the interactions required for microtubule polymerization, according to Avila. Therefore, he and his group carried out limited proteolysis of tubulin by nine different proteases with different specificities. These investigators thus found three main cleavage sites. These exposed sites define four regions (domains) which have been termed I, II, III, and IV beginning from the amino and extending to the carboxy terminal end. By sequence analogy with other related proteins, and from the previous data by other groups, Avila found that I and/or II may contain a nucleotide binding site. Also region II from the alpha subunit and III of the beta subunit appeared to be involved in the dimer interaction, and region IV is an assembly-regulating domain. The binding of the MAP's site on tubulin is mainly by this region. This interaction was found to be modulated by phosphorylation of the tubulin IV region or by the phosphorylation of MAP's. The interaction of MAP's with tubulin or other molecules was also studied by limited proteolysis. These analyses enabled Avila to design a map for some of these microtubule-associated proteins.

Studies of plectin, MAP's, and Dynein-like proteins were presented by G. Wiche (Institute of Biochemistry, University of Vienna, Austria). Plectin is an abundant and widespread cytoskeletal high-molecular-weight polypeptide that is expressed already in early preimplantation embryos. As deduced from circular dichroism, hydrodynamic, and ultrastructural studies carried out by Wiche and his group, plectin is a tetrameric molecule of dumbbell-like structure. Plectin's globular end domains seem to be involved in various interactions of plectin including *in vitro* self-assembly into linear string-of-beads-like structures and cross-linking of intermediate filaments. Using solid-phase binding assays, vimentin, MAP 1, MAP 2 and spectrin-type molecules have been identified by Wiche and coworkers as plectin's interaction partners. Thus, Wiche suggests that plectin is a multifunctional cytomatrix protein engaged in interlinking cytoskeletal filaments of various types and connecting them to the membrane skeleton. Immunolocalization of plectin in various tissues and cells supports this concept. Aside from plectin, Wiche and his group have shown that proteins sharing size and epitopes with MAP 1 and MAP 2 from neuronal cells are widespread in various cell and tissue types. However, unlike plectin, these proteins seem to vary extensively in primary structure, with tissue specificity apparently prevailing over species specificity. Wiche thinks that the structural diversity of MAP's could be important for the proposed function of MAP's as cross-linkers between microtubules and their various interaction partners. In fact, if the binding of MAP's to different partners is specific and dependent on the primary structure of MAP's, these proteins could be vital elements in determining the structure and shape of the cytoplasm. These investigators are now engaged in testing this hypothesis. In addition, Wiche and his very productive group have isolated a novel high-molecular-weight microtubule binding protein from mammalian brain. This new protein appears to share some properties with axonal Dynein, including a relatively high ATPase activity. Thus, according to Wiche, this protein is a candidate for a cytoplasmic variant of Dynein.

4 CYTOSKELETAL ORGANIZATION IN DIFFERENTIATING CELLS AND EARLY DEVELOPMENT

Reorganization of tubulin pools during early mouse embryogenesis was discussed by J. Arechaga (Department of Cellular Biology, University of Granada). Compaction and cavitation are the two major morphological changes in preimplanted mammalian embryos. During these processes a homogeneous population of blastomeres at the 8-cell stage normally lose their totipotency and segregate themselves after the next two cleavages, resulting in specification for inner cell mass and trophectoderm. Several hypotheses have been invoked to explain blastomere determination in relation to the possible role of positional information, blastocyst fluid environment, polarity, and phenotypic differences after cell divisions, changes in cell surface molecules, etc. However, according to Arechaga, only careful analysis and correlations of the many individual cytological and biochemical modifications during compaction and cavitation can lead to a complete interpretation of this crucial period of development. Through studies with different kinds of drugs which selectively affect the cytoskeleton (colchicine, colcemid, taxol, nocodazol) indirect evidence implicating the microtubule system in compaction and cavitation has been obtained. Moreover, there is evidence that calcium-free media reverses the compaction process, which could be related to the very well-known requirement for calcium ions in the regulation of microtubule assembly in the cells.

As an attempt to further understand the molecular aspects of the role of microtubules in mammalian preimplantation embryos, Arechaga and his group examined the intracellular tubulin pools and the internal distribution of their assembled forms. Microtubule cytoskeleton was extracted under stabilizing conditions and in the presence of protease inhibitors. Microtubule pools and free tubulin were determined after separation of the soluble and membrane fractions using both differential and gradient centrifugation in sucrose solutions. The data suggest an intracellular redistribution of microtubules from cytoplasmic to the membrane domain associated with the onset of compaction. Further support of this finding was obtained by electron microscopy of thin sections from mouse embryos and by indirect immunofluorescence using antitubulin antibodies. In addition, a preferential distribution of tubulin in the inner cell mass as compared with the trophectoderm was found. Arechaga thinks that the changes in the distribution of microtubules may respond to cytoarchitectural demands during compaction and cavitation.

Studies on the organization of intermediate filaments in cortical astrocytes in primary culture was presented by J. Ciesielski-Treska (INSERM UNIT 44, Center of Neurochemistry of the CNRS, Strasbourg, France). Intermediate filaments (IF) are constituted from several immunologically and biochemically distinct classes of proteins which are limited to specific cell types and tissues. Within the central nervous system (CNS), vimentin and glial fibrillary acidic protein (GFAP) represent the principal constituents of IF found in astrocytes. Astroglial precursor cells isolated from neonatal rat brain cortex acquire GFAP within several days in culture but, in contrast to their development *in situ*, maintain vimentin. The acquisition of the potential to synthesize GFAP proceeds more slowly in immature astroglial cells cultured under conditions which favor cell migration and proliferation. GFAP is first detected by immunofluorescence in the perinuclear region of differentiating astrocytes, and filaments containing vimentin and GFAP are organized from a common center localized close to the centriolar region. In flat polygonal astrocytes, IF are concentrated around the nucleus and dispersed in an irregular fashion throughout the cytoplasm. In process-bearing astrocytes, IF are organized in a radial fashion in the cell body and oriented along the cell process axis. Double-immunofluorescence labeling with antibodies to GFAP and to tubulin revealed an extensive co-distribution and parallel organization of IF and microtubules in all morphological types of astrocytes studied. Thus, according to Ciesielski-Treska, microtubules in conjunction with IF are responsible for morphological changes which characterize the formation of mature process-bearing astrocytes.

Ciesielski-Treska and her group also found that vimentin and GFAP are resistant to extraction with low-salt buffer containing nonionic detergent independently of the organization of IF. Extraction of astrocyte proteins in the presence of millimolar free calcium caused the degradation of both vimentin and GFAP. The proteolytic breakdown products have a lower isoelectric point and become soluble in extraction media. Calcium introduced into digitonin-permeabilized astroglial cells at micromolar concentration produces a limited proteolysis of vimentin and GFAP. According to these investigators this process, which is influenced by cyclic AMP and phorbol ester involved in the stimulation of protein kinase C, may represent a posttranslational modification of vimentin and GFAP rather than the initial step of their degradation. These results indicate that, in addition to their structural role, IF containing vimentin and GFAP may represent an important component of the receptor-linked response systems operating in astrocytes.

Studies on the free intermingling of tubulin isotypes in the assembly of functionally distinct microtubules was discussed by N.J. Cowan (Department of Biochemistry, New York University Medical Center). In mammals, α- and β-tubulins are each encoded by multigene families. About two-thirds of the sequences contained in these families are pseudogenes bearing the hallmarks of cytoplasmic messenger RNA's (mRNA's); the remainder are expressed in distinct developmental patterns that are in some cases tissue-specific. Thus far, six expressed mammalian α-tubulin genes and six mammalian β-tubulin genes have been identified, with the exception of a single pair of α-tubulin genes, and each encodes a unique α- or β-tubulin isotype, distinguished from other α- and β-tubulin gene products by one or more amino acid substitutions. The existence of multiple tubulin isotypes has led to speculation that the functional diversity of microtubules might be a result of the subcellular sorting of these isotypes, so that distinct kinds of microtubule might be assembled from or enriched in one or more α- or β-tubulin gene products. On the other hand, according to Cowan, the existence of distinct but closely related tubulin isotypes could be unrelated to microtubule function, and reflect instead the need for quantitative regulation of tubulin synthesis in different cell types and at different stages of differentiation.

To examine whether different isotypes are segregated into functionally distinct microtubules, Cowan and his group generated immune sera that are capable of discriminating among the various naturally occurring β-tubulin isotypes. Cloned fusion proteins encoding each isotype were used first to tolerogenize animals against shared epitopes and then as immunogens to elicit a specific response. Western blots of cloned fusion proteins and cell and tissue extracts as well as immunofluorescence analysis of fixed microtubules confirmed the absolute isotype specificity of the resulting sera. In experiments using these sera, Cowan and coworkers showed that there is neither complete nor partial segregation of β-tubulin isotypes: both interphase cytoskeletal and mitotic spindle bundles are mixed copolymers of all expressed β-tubulin isotypes. Indeed, a highly divergent isotype normally expressed only in certain hematopoietic cells is also indiscrimantly assembled into all microtubules both in their normal context and when transfected into HeLa cells.

5 THE CYTOSKELETON IN DEVELOPMENTAL NEUROBIOLOGY

A report on the characterization of a microtubule assembly promoting factor (NbMAPf) was presented by N.W. Seeds (Department of Biochemistry/Biophysics/Genetics, University of Colorado School of Medicine, Denver). This novel factor had been discovered by Seeds and his group. The factor is a protein with a molecular weight of 160,000 and is heat and trypsin labile. The NbMAPf promotes assembly of purified brain tubulin into microtubules. The assembly-promoting activity does not cycle with microtubules (MT's) during assembly and additional cycles of assembly require the readdition of the supernatant fraction from the previous assembly, suggesting that the factor may play some catalytic role or be inactivated during the assembly process. Apparently, purification of the factor has been difficult, and only a 70-fold increase in specific activity has been obtained thus far. Seeds and his group prepared antibody to their enriched preparation of the factor and found that the rabbit antibody did not affect several components of the microtubule system, indicating that NbMAPf is a unique protein involved in MT assembly associated with neurite outgrowth.

The investigation of Tau proteins during neuronal differentiation was reported by J. Nunez (INSERM UNIT 282, Hospital H. Mondor, Créteil [Paris], France). Most of the major MAP's (MAP 1, 350 kDa, MAP 2, 300 kDa and Tau, 52-65 kDA) undergo quantitative or qualitative changes in expression during brain development. These changes suggest that MT's in developing axons and dendrites differ in composition from their counterpart in mature neurons. Nunez and his group have used several experimental approaches to better characterize immature Tau and to understand the functional significance of the changes in Tau composition seen during brain development. The results of their studies showed that immature Tau is localized in growing axons whereas adult Tau might be responsible for the stabilization of adult axonal connections.

Studies of cytoplasmic and extracellular control of neurite formation were presented by A. Krystosek (University of Colorado Health Sciences Center, Denver). Differentiation of the neural hybrid cell line (NG 108-15) was studied with the aim of elucidating the mechanisms regulating neurite formation and growth. Few cells proliferating in serum-containing medium possess neurites, whereas neurite formation occurs spontaneously in serum-free medium. The percentage of neurite-forming cells and the number of neurites per cell can be further increased in response to dibutyryl cyclic AMP. Organization of tubulin and the number and placement of the microtubule organizing centers (MTOC's) were explored during these different growth behaviors using indirect immunofluorescence with anti-tubulin antibodies.

The results of Krystosek's study indicated that undifferentiated NG108-15 cells contained a loose network of cytoplasmic microtubules, whereas a dense packing of these polymers was present in the neurites of maturing cells. Under all growth conditions, greater than 90 percent of the cells possessed a single MTOC. This structure was thus unaffected by either proliferative state or the number of neurites elaborated per cell. Several additional experiments were carried out indicating that neurite formation by NG108-15 cells is under several levels of control. In the culture environment, microfilament-containing filipodia appeared to participate in the transduction of extracellular information mediating the directional choice for neurite initiation. According to Krystosek, the intracellular organization for microtubules appears to be a secondary structural response facilitating extension of neurites.

6 INVOLVEMENT OF INTERMEDIATE AND ACTIN FILAMENTS IN CELLULAR DIFFERENTIATION AND CANCER BIOLOGY

A study of intermediate filament-like proteins in *Drosophila melanogaster* was presented by R. Marco (Department of Biochemistry, University of Madrid, Spain). The function of the intermediate filaments, the third highly insoluble element of the cytoskeletal framework of the cells, remains to be established in spite of much effort invested in this problem. Marco and his group were interested in the possibility of exploiting the genetic and developmental versatility of *Drosophila* in the clarification of the role of these components. In inital experiments they were unable to detect intermediate filaments (IF) in *Drosophila* using antibodies prepared against mammalian IF. Therefore, they decided to rely on the two major properties of these cytoskeletal components, namely, their insolubility and their ability to polymerize into filaments detectable by electron microscopy. Using a purification scheme based on these properties, Marco and coworkers were able to obtain a set of polypeptides from adult *Drosophila melanogaster* (fruit fly) which showed the properties of IF. These polypeptides were found to be present although in much reduced concentrations in extracts from embryonic stages. The biochemical properties and developmental profiles of this set of polypeptides were then studied. A doublet of 81 kDa and 83 kDa present in their purification fractions are considered by Marco to be candidates for being the *Drosophila* adult laminas. Currently, Marco and his group are producing poly- and monoclonal antibodies against these polypeptides to further characterize their tissue and cellular distribution and organization.

Studies of the expression of genes coding for IF proteins in mouse teratocarcinoma cells was presented by D. Paulin (Pasteur Institute and University of Paris, France). Embryonal carcinoma (EC) cells and differentiated derivatives grown in tissue culture have been used by Paulin and her group as a model to study the regulation of cytoskeletal proteins. The distribution of different IF types was examined with specific antibodies able to distinguish vimentin, desmin, keratin, GFAP, and neurofilament polypeptides. Twelve EC lines were shown to express vimentin when cultured as monolayer. One species of vimentin mRNA of 2 kilobases (Kb) was characterized with a cDNA probe in EC cells (PCC3, PCC4, PCC7, and F9). The abundance of vimentin was correlated with the quantity of mRNA present in the cells. When PCC3 were grown as aggregates, the vimentin IF are repressed. *In vitro* differentiation of EC cells can be induced by growth in the form of cell aggregate, confluency, or induced with drugs such as retinoic acid. These investigators followed the organization and synthesis of the tissue-specific IF during the differentiation of EC cells. The onset of neurofilament (NF) expression was found to be concomitant with axon elongation. Neuroepithelial cells contained both vimentin and the 70 kDa NF. Fully differentiated neurons also contain this NF but no vimentin. During myogenic or endodermic differentiation, the switch of vimentin synthesis was observed and the desmin or keratin filaments are synthesized. In terminal differentiation *in vitro*, the final steps appear to closely resemble observations during normal neuronal development.

Changes in the organization of vimentin-type IF during retinoic acid (RA)-induced differentiation of embryonal carcinoma cells was studied by L.C. Moscinski (Department of Pathology, University of Colorado Health Sciences Center). He and his group examined EC cells with antivimentin antibodies using indirect immunofluorescence. Induction of differentiation with RA resulted in an observable alteration in specific vimentin immunofluorescence within 48 hours and a progressive appearance of characteristic wavy filamentous structures throughout the cytoplasm of most cells within 6 days. Two-dimensional gel analysis of proteins from Triton-insoluble cytoskeletons of EC and RA-treated EC cells indicated that the changes in vimentin organization were accompanied by an increased amount of vimentin relative to other cellular proteins. This increase in the amount of vimentin was not accompanied by any detectable alteration in phosphorylation or sensitivity of filament phosphorylation to cyclic AMP. Vimentin filaments were also studied at the electron microscopic level using the peroxidase-labeled antibody technique. Examination of EC cytoskeletons using both scanning and transmission electron microscopy demonstrated a fine network of vimentin filaments, frequently with concentrations near the nucleus. Studies of similar preparations from EC cells at various times following RA treatment revealed an increase in the number and size of filament bundles extending through the cytoplasm. Thus, these results are consistent with the hypothesis that IF proteins are developmentally regulated and may be temporally related to changes in cytoplasmic organization during differentiation.

A review of research on the developmental basis of cancer was presented by G. B. Pierce (Department of Pathology, University of Colorado Health Sciences Center). Pierce stated that carcinomas may be conceptualized as caricatures of the process of tissue renewal. They originate from the undifferentiated determined stem cells of normal lineages and they are composed of malignant cells, and partially and in some tumors, completely differentiated progeny of the malignant stem cells. The caricature results from an overproduction of maligant cells in relationship to the few that differentiate. Differentiation of malignant cells to benign cells is not a reversal of the malignant process. Reversal of the malignant process would require a malignant stem cell to become a normal stem cell. This was demonstrated by placing embryonal carcinoma cells of the mouse into blastocysts, and placing of the blastocysts in the uteri of surrogate mothers. A chimeric mouse was born, indicating that the malignant stem cell was incorporated into the developing embryo and it and its offspring responded to environmental controls. Regulation of the embryonal carcinoma cell is mediated by contact with the blastocyst in the presence of blastocele fluid. Neuroblastoma cells are regulated in terms of tumor formation when placed in either the neural crest migratory route or in the anlagen of the adrenal medulla. The mechanism of this regulation is unknown. Regulation of tumor and colony formation of melanoma cells occurs when the cells are placed in the embryonic skin on the day that melanocyte precursors arrive in the skin. The effect is mediated by a soluble small-molecular-weight factor synthesized by the embryonic cells. In addition to embryonal carcinoma, neuroblastoma, and melanoma, leukemia cells have been shown to be regulated in the 10-day mouse placenta. Thus, Pierce concludes that if four out of four tumors tested in the appropriate embryonic fields fail to produce tumors in expected number, that there is an embryonic field capable of regulating each malignant tumor which may have important prospects for therapy.

7 CYTOSKELETAL INTERACTION IN DEVELOPMENT

Studies of the structure and cell-type specific expression of the cytokeratin multigene family were presented by J.L. Jorcano (German Cancer Research Center, Heidelberg, West Germany). The cytokeratins are a family of approximately 20 polypeptides which constitute the cytoskeleton of IF (8-12 nm diameter) present in epithelial cells. Based on biochemical, immunological, and sequence data, they have been divided into two subfamilies: the basic (type II) and the acidic (type I) cytokeratins. Heterotypic tetramers contain two molecules of each type and a[illegible] the structural subunits of cytokeratin IF. Different epithelia can be characterized by the combinations of basic and acidic cytokeratins they synthesize. In addition, complex epithelia can synthesize specific cytokeratins in different layers or regions of the tissue. Therefore, these proteins are excellent markers for the different routes of epithelial differentiation. Jorcano and his group have cloned the genes for several cytokeratins in order to understand the mechanisms governing the control of their cell type-specific expression. These genes share a common intron-exon pattern, suggesting that they have evolved from a common ancestral gene. Genomic walking experiments indicate that many of the genes encoding basic cytokeratins are close together, but probably separated from those coding for acidic cytokeratins. However, this arrangement seems not to be related to their expression during development and differentiation because neighboring genes are coexpressed in some tissues, are differentially expressed in others, or can exhibit totally different patterns of expression. The 5'-flanking regions of cytokeratin genes lack extensive homology. The only motif consistently conserved is an AAPuCCAAA box found upstream of the TATA box in the genes coding for epidermal cytokeratins, but absent in genes expressed in simple epithelia. These 5'-flanking regions are, however, highly homologous in genes coding for the same cytokeratin in different species, indicating that this evolutionary conservation could be important for the regulation of the cell type-specific expression of these genes. In fact, according to Jorcano, biological tests have confirmed this hypothesis.

8 CONCLUSION

This intensive and very interesting conference covered the molecular, cellular, and genetic basis of the role of the cytoskeleton in cell differentiation and development. The broad area covered by this topic stresses the importance of the interdisciplinary bridges connecting modern cell biology and biochemistry in experimental embryology. Enormous progress has been made in this area due to a large extent to the excellent techniques now available such as immunological techniques, recombinant DNA technology and sophisticated methods for protein purification and analysis. The presentations focused on the analysis of the assembly dynamics of microtubules, intermediate filaments, and actin filaments to provide the structural basis of the role played by the cytoskeleton in the differentiation of a variety of cell systems, early embryogenesis, and to the biological and genetic aspects of cytoplasmic organization.

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